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## (54) Imaging immunoassay detection system and method.

(57) Light generated by one or more chemical reactions in a discrete array is determined, both as to emissions per reaction and as to relative location of the reactions, e.g. using a system comprising:

a sample carrier having spaced-apart discrete areas each containing a reactant capable of emitting light photons if a reaction takes place;

a photon receiver, for receiving photons emitted from each reaction simultaneously; and

a signal generator coupled to the photon receiver, for generating a signal representing the x-y location of each reaction.

The signal generator may be a photomultiplier, charge-coupled device or vidicon tube. The invention is particularly suited to the determination of reactions and non-reactions, e.g. between an array of labelled antibodies and a specimen potentially containing one or more complementary antigens, in an immunoassay.

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This invention relates to an imaging immunoassay detection system and method.

BACKGROUND OF THE INVENTION

Highly sensitive instrumentation for immunoassay techniques haS been developed to enable measurement of reactions of extremely small quantities of biological and chemical substances. For example, instruments for radioimmunoassay are used which are sensitive, accurate and precise, but require expensive gamma-counting equipment. Other disadvantages of such systems include the short half-life of the radioisotopes, and the danger of using and disposing of the radioactive compounds used in such assays.

Another prevalent technique is the colorimetric enzyme immunoassay which utilises enzymes as labels. An enzyme-linked immunoreactant binds either to an antigen or to an antibody, causing a reaction which yields a quantitative measure of the antibody or antigen, which can be detected by a colour change. Such an assay is usually slower than other conventional techniques involving automated assays.

A third method that can be used is a fluorescence immunoassay, based on the labelling of an antigen or antibody with fluorescent probes. US-A-4320970 discloses a photon-counting fluorimeter that may be used in such an assay. Disadvantages of such a system include the necessity of processing only one sample at a time. Other systems attempt to use laser beams as the external light source to excite the solution, as disclosed in US-A-3984533. Again, this system can process only one sample at a time.

Instrumentation for luminescence assays advantageously involves a self-exciting luminescing system, in direct contrast to fluorimeters which utilise an external light source. In general, existing

luminometers are complex in operation and require the use of substantial quantities of the reagent being sampled.

Further efforts to analyse more than one reagent sample simultaneously, in a quantitative sense, have not been successful. Efforts toward this end are illustrated by a system described by Schroeder et al in "Immunochemiluminometric Assay for Hepatitis B Surface Antigen", Clinical Chemistry, Vol. 27 No. 8 (1981), wherein a carrier is prepared containing a plurality of reagents for analysis by a luminometer which measures light production during reaction by photo-counting. However, this method and apparatus have the disadvantage of requiring the reactions to be measured sequentially, one at a time. A microprocessor was used to control fluid and air valves for adding the desired chemicals to each well; the carrier was moved in an x-y plan, to position the individual wells sequentially over a phototube, in order to enable the photons emitted by the reaction to be counted. The results were displayed by a printer. Even though photons were counted for 2 seconds at 10 second intervals, obviously a great deal of time would be required to analyse hundreds or thousands of test specimens in sequential order, one at a time.

In addition, GB-A-2132347 discloses a chemiluminometer for simultaneously handling multiple samples. The results obtained, however, are only semi-quantitative.

It has heretofore not been possible to carry out luminescent assays on multiple, small volume samples simultaneously in a short period of time, i.e. seconds. Presently existing technology permits only one such assay at a time and often requires large volume samples, i.e. of 200 µl or more.

As used herein, the terms "luminescent" and "luminescence" mean all kinds of light emission except

incandescence and include chemiluminescence, bioluminescence, prompt fluorescence, delayed fluorescence and phosphorescence and the like.

Rees et al, J. Phys. E: Sci. Instrum. 14 (1981) 229-233, describe a miniature imaging photon detector with a transparent photocathode. It is proposed for use in astronomy and geophysics.

SUMMARY OF THE INVENTION

The present invention overcomes the given drawbacks and provides an imaging immunoassay detection apparatus system and method capable of detecting and quantifying multiple light-emitting reactions from small volume samples simultaneously.

The present invention is very advantageous inasmuch as it is very rapid because it analyses all samples simultaneously, is extremely accurate because it requires no mechanical motion of components, has no repositioning errors as in sequential resolution, can use an internal standard such as a known sample to obtain comparative information, is adaptable with a filter to handle any particular wavelength of light, and is versatile in that it can detect assays requiring external light as well as those that do not require external light, thus being able to operate with immunoassays utilising luminescence and fluorescence and the like.

In brief, the present invention comprises an imaging system for detecting photons generated by chemical reactions comprising sample carrier means having a plurality of individual areas each containing individual chemical reactant samples capable of emitting photons if a reaction takes place, the plurality of reactant-containing areas being arranged in spaced relationship with respect to each other; imaging means associated with said carrier means for simultaneously receiving individual photons emitted from each area sample where a

reaction is taking place; and means coupled to the photon-receiving imaging means for generating a signal representing the x-y location of each area sample generating a photon, whereby the reactants in each area sample having a reaction and the number of its photon emissions over any predetermined period of time may be simultaneously identified.

The invention also comprises a method of simultaneously detecting photons generated by a plurality of chemical reactions, comprising the steps of providing a plurality of individual chemical reactant samples each capable of emitting photons when a reaction takes place, the samples being arranged in spaced relationship with respect to each other, and simultaneously detecting the presence and x-y location of each photon emitted from any reacting samples, whereby the total number of photons emitted from each reacting sample over a predetermined period of time may be determined.

Thus, the present invention is not only extremely rapid and processes simultaneously multiple assays, but also enables considerable economies to be made in the use of often expensive reagents concerned because only small quantities of samples are required.

DESCRIPTION OF THE DRAWINGS

Figure 1 is a diagrammatic representation of the novel photon detection system;

Figure 2 is a diagrammatic representation of the imaging photon detector used in the system of Figure 1;

Figure 3 is a representation of both background noise signals and signals from discrete areas of reaction (whereby the background noise signals may be cancelled, leaving only reaction signals);

Figure 4 represents a carrier in which multiple antibody labels may be used simultaneously or in which a standard reaction may be compared with other reactions;

Figure 5 is a diagrammatic representation of how a wavelength interference filter may be used with the carrier of Figure 4 to pass only a particular light wavelength, thereby allowing only photons of a particular wavelength corresponding to a labelled antibody to pass to the imaging detector;

Figure 6 is a schematic representation of a circuit in a microprocessor for subtracting background noise from the sample signals to obtain an output signal representing substantially only pure photon emission from a reactant sample; and

Figure 7 is a diagrammatic representation of the use of invisible ultraviolet radiation as a source of external light.

DETAILED DESCRIPTION

The apparatus of the present invention, which will be described in further detail below, may be utilised with any number of different assay techniques as stated earlier, but will be described with particular reference to the use of luminescent immunoassays in the detection of antigen-antibody reactions. More particularly, the invention can be employed to detect the characteristic reactions of labelled monoclonal and polyclonal antibodies with antigens found in samples such as urine, faeces, blood, milk and water and the like.

Polyclonal antibodies are well-known. Monoclonal antibodies may be prepared by the technique first described by Kohler and Milstein, Eur. J. Immunol. 6, 292 (1975). In order to detect the presence of particular antigens, the monoclonal antibodies may be labelled with a multitude of different labels, such as luminescent or fluorescent compounds. Further, the particular labels utilised in the present invention must be capable of emitting light once the antigen-antibody reaction occurs, and thus the reactions are designated as "light-emitting

reactions". The present invention will be described in general with reference to a luminescent-labelled monoclonal antibody, although fluorescent labels may also be used as disclosed hereafter. As used herein, the term "reactants" means the combination of (1) a monoclonal antibody labelled with a luminescent or fluorescent compound, and (2) an antigen.

Luminescence is the emission of light by an atom or molecule as an electron is transferred to the ground state from a higher energy state. In both chemiluminescent and bioluminescent reactions, the free energy of a chemical reaction provides the energy required to produce an intermediate reaction or product in an electronically excited state. Subsequent decay back to the ground state is accompanied by emission of light. Bioluminescence is the name given to a special form of chemiluminescence found in biological systems such as the firefly, in which a catalytic protein or enzyme, such as luciferase, increases the efficiency of the luminescent reaction. When this luciferase enzyme is combined with its substrate, luciferin, in the presence of ATP (adenosine triphosphate), magnesium and oxygen, a flash of light is produced, whose intensity is proportional to the amount of ATP present in the sample. The firefly luciferase/luciferin/ATP system is as follows:

$$ATP \xrightarrow[\text{Luciferin}]{\text{Luciferase}} AMP + PP + h\nu$$

where $h\nu$ is the energy of a photon, h is the Planck constant, and $\nu$ is the frequency associated with the photon.

Assays of the invention can directly determine the number of live organisms in a sample, either because the presence of ATP in a test sample indicated live cells, or because of the presence of immunoglobulins labelled with

a luminescence-detectable enzyme (like peroxidase or luciferase).

Chemiluminescent substances such as luminol may also be utilised in a horseradish peroxidase-catalysed oxidation, as follows:

$$\text{luminol} \xrightarrow[H_2O_2]{\text{peroxidase}} \text{products} + h\nu$$

In the present invention, the "light-emitting reactions" generate photons which are coupled to an imaging device such as an imaging photon detector, a charge-coupled device, or a vidicon tube (any of a variety of camera tubes having a photoconductive target). In the preferred embodiment, an imaging photon detector is used.

In particular, the reactions may be generated by reactants spatially arranged in individual areas on a sample carrier in a single row or column or by a two-dimensional array of reactants spatially arranged, for instance, in rows and columns. For example, a carrier may have an array, such as rows, of 1 mm outside diameter nylon tubes containing the labelled monoclonal antibodies, to which is added the specimen or specimens being tested for the presence of an antigen. The fluids involved are self-contained and of a very small volume. Thus, an advantage of the present invention is that the imaging photon detector can quantify (in 10 seconds or less) light emitted from multiple "light-emitting reactions", in volumes of 3 µl or less, i.e. much smaller than can be used in known apparatus.

Another carrier suitable for use with an imaging photon detector is a microtiter plate with multiple samples in rows and columns. A particular plate may contain as many as 96 individual wells. Each well contains different labelled monoclonal antibodies adsorbed on the surface of the plate. A portion of the specimen is added to each well. The presence and

quantity of a particular antigen in an individual well is determined by the number of photons generated by the antigen-antibody reaction.

A third carrier involves the principle of using immobilised antibodies on a plurality of filaments; labelled monoclonal antibodies are immobilised in individual areas on a plurality of filaments. Each filament may bear a different labelled monoclonal antibody capable of emitting light upon the detection of an antigen. Because the reactions on the individual filaments generate light, the imaging photon detection system can quantitatively determine the presence of particular antigens.

The present invention envisages the use of any number of different types of multiple, chemically-produced, light-emitting reactions which can be imaged by the image photon detector. As the samples emit light, the present invention counts the individual photons impinging upon a light sensitive photocathode of an imaging photon detector.

Each of the above means for containing a plurality of reactants can be used in the apparatus system described below, in which the container is identified as the specimen carrier means.

The apparatus system will be described with reference to Figures 1 and 2.

Fig. 1 is a system for quantitative assay analysis of multiple biochemical images using an imaging photon detector. The system enables the detection of very low concentrations of substances present in fluid samples or specimens which, in the course of their reaction, emit light photons under certain conditions. In particular, the system has demonstrated sensitivity in the order of $10^{-16}$ and lower.

Fig. 1 shows a specimen carrier means 10 which may include a plurality of fluid samples all capable of simultaneously undergoing a reaction. Samples can be spaced in individual areas as a row or column or in a two-dimensional array of rows and columns as shown in Fig. 3 and Fig. 4, for example only. The reactions that produce light generate photons 12 which are focused by an optical system 14 to form the image of the light outputs of each of the samples on a photoconductive target forming a portion of an imaging photon detector (IPD) 16. The imaging photon detector 16 will be disclosed in detail hereinafter but is known in the art; it immediately converts incoming light into quantitative information which can be stored and processed within a memory of any conventional computing means such as a microprocessor 24.

The imaging detector 16 of the present invention takes simultaneous readings of discrete sample areas such as the solid dark areas of Fig. 3, rather than averaging the readings of the entire sample area (including the carrier area surrounding the sample). Background noise, represented by the shading lines in Fig. 3, is caused by non-specific binding antigens or antibodies to the solid surface of the carrier, which are not washed away in the preparation of the carrier. Conventional detectors read these signals generated by this undesirable binding and, because these conventional detectors average the signal over the entire sample area, they interpret these undesired false signals as a positive reaction. Background noise effectively decreases the sensitivity of the assay at relatively low levels of concentration, where the positive reaction signal has nearly the same intensity level as the background noise.

The present invention eliminates the background noise problem by simultaneously reading the signal from the background environment and the signal from the

concentrated reaction area and comparing the two readings. Because the present imaging immunoassay detection system can read signals from numerous discrete reaction areas at the same instant, a real time measurement of the signals from the discrete areas of reaction in a two-dimensional array can be taken, averaged, and compared with the signal representing the background noise caused by the non-specific binding. The computer 24 can analyse and display the results by subtracting from the signals representing the discrete areas of reactions the signals representing the background noise, as shown in Fig. 6, thereby leaving the pure reaction signals.

Further, since the present system can simultaneously evaluate a carrier, such as a 96-well microtiter tray, without repositioning the carrier or tray as in systems using sequential detection, resolution errors that occur from imprecise mechanical repositioning of the samples between measurements as required in the prior art are eliminated.

Since the present imaging immunoassay detection system can look at more than one discrete reaction at a time, contrasting reactions can be analysed relative to one another. By way of illustration, in a two-dimensional array of samples as in a microtiter tray represented in Fig. 4, reactions can be compared side-by-side simultaneously. The amount of photons generated by each reaction can be read and analysed by computer 24 and the relative extent of reaction compared. In this way, a more accurate comparison can be made between specific samples, thus providing better test results. As an example, a negative reaction may be placed in a discrete area A in Fig. 4, to serve as a control for purposes of comparison with a positive reaction in a discrete area B. The negative reaction in

discrete area A may still generate spurious signals caused by non-specific binding, as pointed out earlier. This background noise level is potentially constant across a given carrier such as a microtiter tray and is useful in setting up a base level of signal generation from which more positive reaction can be compared.

Thus, the present imaging system is capable of rapid quantitative analysis of samples. Because of its unique ability to simultaneously read and analyse numerous samples, the time necessary to produce results is dramatically reduced.

Furthermore, the sensitivity of the present imaging system allows for very accurate measurements even at very low concentrations of the samples. For instance, an imaging photon detector is capable of measuring individual photons of light. By using amplifiers, the system is able to register very low concentrations of materials and is therefore useful in areas such as diagnosing for the presence of infectious organisms, as well as drug monitoring and disease detection.

Because of its sensitivity, the imaging system can not only detect minute quantities of a reaction samples, but can also read a very small area of reaction. Thus, the amount of reagent and the area which are needed to conduct the assay are less then before, thereby minimising the cost of reagents and carrier materials.

The output of the imaging photon detector 16 on line 18 comprises analog signals which represents the x-y spatial correspondence of each detected photon, thus identifying electrically the x-y address of the sample or specimen that produced the light. These analog signals are coupled to an analog-to-digital (A/D) converter 20 which produces digital output signals on lines 22 representing the spatial orientation of the specimen source producing the photon received by the imaging

photon detector 16 and thus identifying the particular sample or specimen which produces the photon. The digital signals 22 are coupled to a microprocessor 24 which stores and analyses the image information and can be programmed to display it in any desired format. The reactions continue to generate light throughout any desired predetermined period of time, e.g. as little as 10 seconds or less, and the number of photons produced by each reacting sample is accumulated in the memory of microprocessor 24. Thus, microprocessor 24 produces signals on line 26 to video display 28 and printer 30 for visual display and analysis of the light received, and accumulated, from samples 10.

A bar chart may be displayed on video terminal 28 which identifies each of the samples and illustrates the relative amount of light or number of photons being generated by each sample. Such a bar chart could also be produced, for a permanent record, by printer 30.

Also, a two-dimensional image of the array of samples as they are physically located (i.e. their x-y address) can be produced, the intensity of the light generated by each sample being indicated either in colour or by numerals, thus identifying which sample is generating the greatest amount of light. Obviously, the microprocessor 24 can perform any operation on the samples as desired to correct and calibrate the image and to compensate for any inherent noise in the system such as by subtracting the background noise, as explained earlier. Noise also may be reduced by cooling the imaging photon detector 16 with a cooling unit 15 in any well known manner such as by circulating a cooling liquid or a refrigerant about the imaging photon detector 16. Cooling the detector 16 reduces the tendency for free electrons to be emitted from elements of the detector 16, and which assist in generating the background noise.

Fig. 2 is a diagrammatic representation of the construction details of the imaging photon detector used in the preferred embodiment herein and which is known in the art. The detector may be type IPDG1 or type PIDF1 manufactured by Instrument Technology Limited in East Sussex, England. The imaging photon detector 16 is a two-dimensional imaging sensor capable of detecting extremely weak radiation, e.g. capable of detecting an ATP content in the sample down to as low as $10^{-16}$ moles/sample. As indicated earlier, that image is produced in analog form which is converted through an analog-to-digital converter 20 to a digital form for use by the microprocessor 24.

Light is composed of individual photons. Each individual photon has an extremely small amount of energy associated therewith. In most common images, the light contains fluxes of millions or billions of photons per square centimeter and per second. Using the imaging photon detector 16, each incoming photon has a high probability of detection by the photocathode 32.

The photoconductive target 32 can thus be thought of as equivalent to a photographic film except that it has a sensitivity of the order of 100 times greater. When a photon strikes the light sensitive photocathode 32, a photoelectron is released from photocathode 32 and is immediately accelerated into a series of microchannel plate intensifiers or amplifiers 34. As a result of the intensification created by microchannel plates 34, a gain in the range of $3x10^6$ to $3x10^7$ electrons is emitted from the rear of each microchannel plate for each incident photoelectron and thus corresponds to each initially detected photon. The combination of the microchannel plates 34 thus enables extremely small amounts of light to be detected.

A resistive anode encoder 36 located immediately behind the microchannel plates 34 translates the electron burst into signals which can be processed easily into a two-dimensional x-y address of the detected photon and thus the sample. Thus, the analog readout of the resistive anode 36 on line 18 in Fig. 1 is used to present a linear x-y registration of each photoelectron event. The read-out through four orthogonal electrodes 38 is suitably processed to provide digital representation of the x-y position of the incident photoelectron (and thus the sample) by analog-to-digital converter 20 and microprocessor 24, both shown in Fig. 1. Thus, by the use of the imaging photodetector 16, the full image of all of the samples 10 in two dimensions is created by integrating the image focused onto the photocathode 32, photo-by-photon. Thus, the present system detects and presents information relating to multiple imaging, presents the information immediately upon the occurrence of the emitted light, detects extremely small amounts of light down to and including a single photon, and quantifies such information for each specific x-y sample or specimen address.

Instead of an imaging photon detector (which is preferred), the system may use a charge-coupled device (CCD) as the imaging device 16. CCD's are well known in the art. They are used in conjunction with an optical lensing system (such as optical system 14 in Fig. 1) which focuses light from the object being investigated (sample array 10 in Fig. 1) on to the CCD. Varying amounts of light from individual samples are incident on individual pixels within the CCD and charge the pixels to different levels proportional to the incident light. Thus the optical information or light from sample array 10 is available in analog form across the pixels of CCD array 16. The analog information is then shifted out

of the CCD and converted to digital form in a well known manner by the analog-to-digital converter 20 and is then coupled to a memory in computer 24 where the various measurement levels and comparisons can be made by appropriate manipulation of the digital information. See "Imaging", The Optical Industry and System Purchasing Directory, 1983, pp. E-72 to E-74.

As is well known in the prior art, the individual pixels within a CCD array are closely spaced and arranged horizontally in rows and vertically in columns so that a given CCD imaging device 16 provides a fixed number of pixels of information. For instance, some CCD's have 320 vertical columns of pixels and 512 horizontal rows of pixels.

CCD's have several characteristics which make them advantageous in the present invention as an imaging device. CCD's are small and rugged and have closely spaced pixels and are therefore useful where, as here, precise measurements are required. They also receive an image by the direct reception of light energy without being scanned, and store the received data until the data are transferred to another storage device. Further, the data received from the CCD can be processed by simple comparison or detection techniques, thereby avoiding complex and major time-consuming sampling techniques ordinarily used to process such data.

Another imaging device which can be used instead of an imaging photon detector or a CCD is a vidicon tube. The name vidicon is generally applied to any of a variety of tubes having a photoconductive target. The vidicon operates in a well known manner and utilises an electron beam to scan a light-sensitive photoconductive target. A transparent conductive layer applied to the front side of the photoconductor serves as the signal or target electrode. The target electrode is operated at a

positive voltage with respect to the back side of the photoconductor which operates at cathode (near zero) voltage. In operation, the scanning beam initially charges the back side of the target electrode to cathode potential. When a light pattern (light photon) is focused on the photoconductor, its conductivity increases in the illuminated areas and the back side of the target charges to more positive values. The electron beam then reads the signal by depositing electrons on the positively charged areas, thereby providing a capacitively coupled signal at the signal output electrode. See "Imaging Devices", RCA Solid State Devices, 19, pp 3-9.

Thus, as used in Fig. 1, a vidicon 16 receives the photons or light output from the respective reactions through optics 14 and produces the analog output on line 18 as described earlier. The analog output on line 18 is coupled to the analog-to-digital converter 20 from which digital signals are processed in the same manner as for the imaging photon detector.

It is also possible to use multiple antibody labels with the imaging immunoassay detection system of the present invention. Different antibodies are labelled with different indicators and correspond to different discrete areas on the carrier medium. Thus, with regard to Fig. 4, discrete area A may have an antibody labelled with an indicator such as luciferase, discrete area B may have an antibody labelled with an indicator such as a bacterial reductase, and so forth. Since each indicator generates a different wavelength of light, each discrete area for each antibody has its own particular wavelength of light which can be detected by imaging device 16. As can be seen in Fig. 5, a wavelength interference filter 40 of the desired characteristics is placed between samples 10 and optics 14 such that only photons of a

particular wavelength corresponding to an antibody labelled with a particular indicator pass through to the imaging detector 16. In this manner, the novel imaging immunoassay detection system can be made wavelength selective, thereby permitting greater flexibility and sensitivity.

It will be understood, of course, that if a fluorescing material, such as fluorescein, is used as the label, invisible ultraviolet light or black light will have to be used. Fig. 7 illustrates such apparatus, wherein an ultraviolet light source 54 is powered by an appropriate power source 56. Ultraviolet light rays 58 impinge on samples in carrier 10 which fluoresce if a reaction takes place. The remainder of the circuit operates as described above in connection with Fig. 1.

As shown in Fig. 6, the signals detected by imaging device 16, representing background noise and sample signals combined, are stored in a memory 42 of microprocessor 24. The detected signals representing background noise alone are stored in a memory 44 of microprocessor 16. By coupling these two stored signals on lines 46 and 48 to an arithmetic unit 50, and subtracting one from the other an output signal is obtained on a line 52, which represents substantially only pure photon emission from any selected reactant sample. Thus, background noise is substantially eliminated or minimised.

The system of the present invention can be used in conjunction with labelled DNA or RNA probes instead of antibodies as a diagnostic tool. DNA probes or RNA probes are specific sequences of amino-acids that are complementary to particular sequences of a sample piece of DNA or RNA. These probe pieces of DNA or RNA can be labelled with an indicator so as to generate a signal, analogous to an immunoassay, and would be contained in a

carrier 10 such as shown in Fig. 1. In use, the DNA or RNA is first removed from a cell or other structure in a sample containing a DNA or RNA sequence. The DNA or RNA is bound to a surface such as nitrocellulose, and is then denatured so that its complementary strands are separated. The DNA or RNA probe, labelled with an indicator, is then added to the sample and, if the specific complementary sequence of the probe is present in the sample, the sample and probe will combine. The unbound label is washed away and the sample containing bound labelled probe is read in the manner described hereinabove with reference to Fig. 1.

Isotope-labelled assays are also within the scope of the present invention. In such an assay, the indicator component is labelled with an isotope, such as phosphorus-32 or iodine-125, which emits gamma radiation. In the performance of a radioisotopic assay, the isotope-labelled component, typically an antibody, is bound to the analyte of interest, the unbound label is washed away from the reaction zone, and the zone is read. The reading of the central reaction zone is accomplished in this embodiment by the incorporation of a phosphor screen. Phosphor screens are well known in the art and are used to convert electron energy into radiant energy. These screens are composed of a thin layer of luminescent crystals, phosphors, which emit light when bombarded by electrons. In the present case, the phosphor screen receives gamma radiation from the labelled analyte and, in turn, emits photons which are received by the detection system. Thus, in Fig. 1, carrier 10 would be the phosphor screen which received electrons from any reaction and emits light 12 which is focused by optics 14 and processed in the manner explained previously. In this manner, gamma particles are converted into detectable photons which are received and processed as

described hereinabove. Other types of gamma-to-photon conversion means are also usable and within the scope of the present invention.

As an illustration of the sensitivity of the IPD array detector system, the well established firefly luciferase/luciferin-based assay for ATP provides a useful reference. Thus, using the standard "Lumac" firefly luciferase/luciferin reagents for the bioluminescent ATP assay in the Lumac Biocounter luminometer, the lower limit for the assay (carried out as recommended by the manufacturer) is set by the "background" count of photons (of about 10/second) typically experienced. This sets the lower limit of the determination at about $5x10^{-15}$ moles ATP per sample. In contrast, using the IPD system described above, the lower limit of the determination, using similar criteria, is set at about $5x10^{-17}$ moles ATP.

The invention will be further illustrated in conjunction with the following Example, which is set forth for purposes of illustration only and not by way of limitation.

Example

Monoclonal antibodies are prepared according to the method of Kohler and Milstein noted above. In particular, an antibody to Shigella is prepared by the procedure described in WO-A-86/00035 and labelled with luminescent compounds such as the firefly luciferase/luciferin/ATP system. These luminescent labelled antibodies are then adsorbed onto the surface of a microtiter tray. Additionally, each well of the tray may contain labelled monoclonal antibodies to different antigens, thus allowing diagnosis for the presence of a wide variety of different antigens at the same time. This microtiter tray thus contains a number of known antibodies at known x-y addresses or areas on the tray.

The tray is then washed with the specimen containing the unknown antigen(s) and an antigen-antibody reaction occurs. Next, the tray is rinsed and unbound antibodies are removed. Then, the tray is placed in the specimen carrier holder of the imaging photon detecting system described above. The presence and amount of the unknown antigen(s) in the specimen are determined by the photons generated by the characteristic antigen-antibody reactions and their x-y location on the microtiter tray.

CLAIMS

1. A system for detecting photons generated by a chemical reaction, which comprises:

a sample carrier having spaced-apart discrete areas each containing a reactant capable of emitting photons if a reaction takes place;

a photon receiver, for receiving photons emitted from each reaction simultaneously; and

a signal generator coupled to the photon receiver, for generating a signal representing the x-y location of each reaction;

whereby each reaction and the number of its photon emissions during a predetermined period of time may be simultaneously identified.

2. A system according to claim 1, wherein the signal generator is a photomultiplier tube having a photoconductive target, a charge-coupled device or a vidicon tube, for producing x-y location output signals representing the light intensity or number of photons emitted by each reaction.

3. A system according to claim 1 or claim 2, which further comprises:

a computer coupled to the signal generator, for receiving and storing each of the signals, whereby the x-y location and number of photons generated by each reaction during the predetermined period of time can be accessed; and

display means coupled to the computer, for presenting a representation of each reaction area and the number of its photon emissions.

4. A system according to any preceding claim, which comprises means for subtracting background noise signals from signals representing reactions, whereby the resulting signal represents substantially only photon emissions from the reactions.

5. A system according to any preceding claim, for comparing a potential reaction under test with another or a standard control, which further comprises:

means for comparing the signals emitted from different reactions.

6. A system according to any preceding claim, wherein the signal generator generates analog signals, and the system further comprises:

an analog-to-digital converter coupled to the signal generator, for converting the analog signals to digital signals; and

a microprocessor coupled to the analog-to-digital converter, which includes storage means having a plurality of locations therein corresponding to the arrangement or array of areas or of reactions, for storing the digital signals.

7. A system according to any preceding claim, in which the signal generator includes a photoconductive target, and the system further comprises:

an optics system for focusing light photons emitted from the reactions on to the photoconductive target, to generate x-y location output signals representing the light intensity or number of photons emitted by each reaction.

8. A system according to claim 7, which further comprises:

a specific wavelength light interference filter positioned between the sample carrier and the optics system, such that only reactions emitting light of that wavelength are monitored by the system for photon emission.

9. A system according to any preceding claim, wherein the discrete areas are in a single row or column, or in a two-dimensional array of rows and columns.

10. A system according to any preceding claim, wherein at least one reactant contains an indicator which, on

reaction, emits light of a wavelength different from that emitted from other reactions.

11. A system according to any preceding claim, wherein each reactant is a labelled monoclonal antibody against an antigen, and the label emits light on reaction between the antibody and the antigen.

12. A system according to any of claims 1 to 10, wherein each reactant is a labelled DNA or RNA probe specific for its complementary sequence in DNA or RNA, respectively, and the label emits light on reaction between the probe and a sample containing the complementary sequence.

13. A system according to any of claims 1 to 9, wherein each reactant is an isotope-labelled antibody, and gamma rays are emitted on reaction between the antibody and an antigen; if desired, the system further comprises a phosphor screen, for converting the emitted gamma rays into light photons.

14. A method of detecting chemical reactions, which comprises detecting photons emitted, on reaction, from spaced-apart discrete areas each containing a reactant capable of emitting photons when the reaction takes place, and simultaneously determining the x-y location of each photon emitted.

15. A method according to claim 14, which further comprises determining the total photon emission of any reacting sample during the given time.

16. A method according to claim 12 or claim 13, which comprises a step or steps corresponding to the function defined for a system according to any of claims 3 to 6.

17. A method of detecting chemical reactions, by using a system according to any of claims 1 to 13, which comprises introducing an analyte on to the discrete areas of the sample carrier, wherein one of the analyte and the sample carrier contains the said reactant and the other is under test to determine whether or not it contains a component which reacts with the reactant.

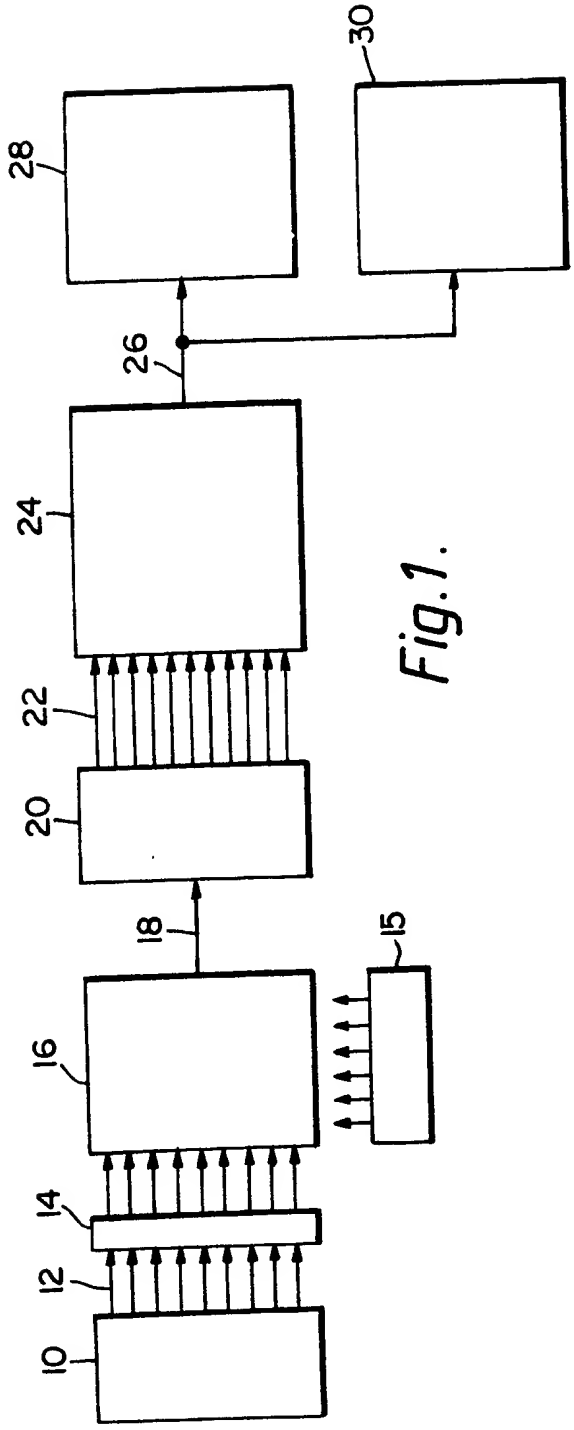


Fig.1.

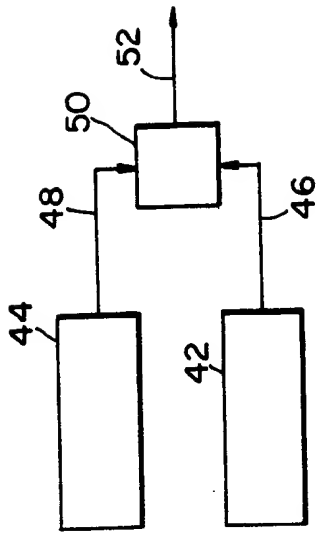


Fig.6.

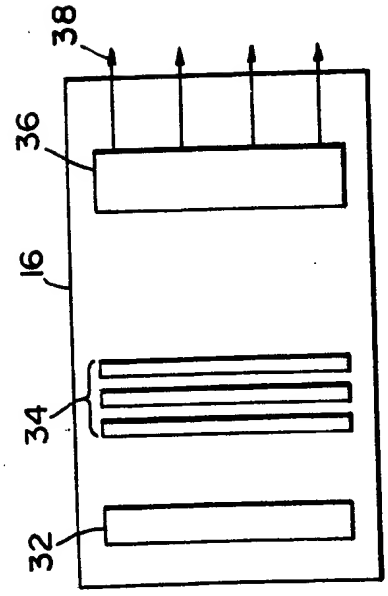


Fig.2.

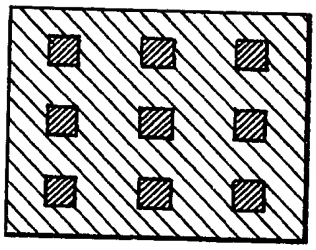

Fig.3.

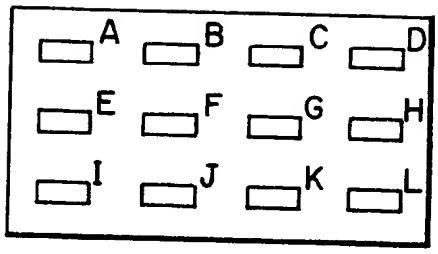


Fig.4.

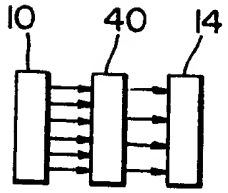


Fig.5.

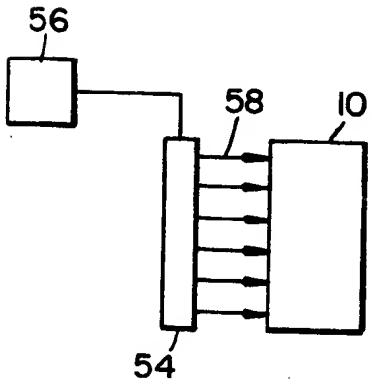


Fig.7.

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(54) **Imaging immunoassay detection system and method.**

(57) Light generated by one or more chemical reactions in a discrete array is determined, both as to emissions per reaction and as to relative location of the reactions, e.g. by using a system comprising:

a sample carrier having spaced-apart discrete areas each containing a reactant capable of emitting light photons if a reaction takes place;

a photon receiver, for receiving photons emitted from each reaction simultaneously; and

a signal generator coupled to the photon receiver, for generating a signal representing the x-y location of each reaction.

The signal generator may be a photomultiplier, charge-coupled device or vidicon tube. The invention is particularly suited to the determination of reactions and non-reactions, e.g. between an array of labelled antibodies and a specimen potentially containing one or more complementary antigens, in an immunoassay.

EP 0 194 132 A3

European Patent Office

# EUROPEAN SEARCH REPORT

0194132

Application Number

EP 86 30 1523

## DOCUMENTS CONSIDERED TO BE RELEVANT

| Category | Citation of document with indication, where appropriate, of relevant passages | Relevant to claim | CLASSIFICATION OF THE APPLICATION (Int. Cl.4) |
|---|---|---|---|
| X | EP-A-0 025 350 (DYNATECH) * abstract; page 8, line 34 - page 10, line 26; page 12, complete: claims 1-3 * | 1,7,9, 10 | G 01 N 21/76<br>G 01 N 33/53 |
| A,P | EP-A-0 148 736 (WALLAC OY) * complete * | 1 | |
| A | EP-A-0 110 610 (WHITEHEAD et al.) * abstract; figure 1; page 18, line 33 - page 20, line 25 * & GB - A - 2 132 347 (Cat. D) | 1 | |
| A,D | US-A-4 320 970 (DOWBEN et al.) * complete * | 1 | |

TECHNICAL FIELDS SEARCHED (Int. Cl.4)

G 01 N 21/00
G 01 N 33/00

The present search report has been drawn up for all claims

| Place of search | Date of completion of the search | Examiner |
|---|---|---|
| BERLIN | 10-04-1988 | GREEN C.H. |

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